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Transcriptional activation by AP-2α is modulated by the oncogene DEK

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ABSTRACT

Cell differentiation and development are highly regulated processes at the transcriptional level. One of the main transcription factors that regulate these processes is AP-2α, a cell-type specific protein required for vertebrate development and embryogenesis. AP-2α also regulates apoptosis and cell-cycle specific events by interacting with the oncogene c-Myc. In searching for novel AP-2α-interacting factors, using an affinity chromatography approach, we have observed that oncoprotein DEK interacts with AP-2α *in vitro*. The existence of an interaction between AP-2α and DEK in cellular cultures was demonstrated by expression of a tagged AP-2α form followed by immunodetection. By transient co-expression experiments using a reporter for *APOE* promoter activity we have found that DEK stimulates the transactivation activity of AP-2α over *APOE* promoter. Finally, electrophoretic mobility shift assays suggested that DEK enhances the DNA-binding activity of AP-2α. Our data suggest a novel cellular function of DEK as a transcriptional co-activator.

INTRODUCTION

The family of AP-2 transcription factors is composed of four members: AP-2α (1,2), AP-2β (3), AP-2γ (4) and AP-2δ (5). Spatially and temporally restricted expression patterns of these proteins (6–8) provide essential clues for embryonic development (9,10), regulation of programmed cell death (11), and cell growth and differentiation (12).

Recently, the relevance of protein–protein interaction on AP-2α activity has been reported. AP-2α modulates the function of Myc as a proliferative agent and also as apoptosis inductor (13). Other known AP-2α-interacting proteins are the retinoblastoma Rb protein (14,15), the Yin Yang 1 factor (YY1) (16), and the transcription factors YB-1 (17) and Sp-1 (18).

We have recently described the presence of allelic polymorphisms in the transcriptional regulatory region of the *APOE* gene, which produce variations in promoter activity (19). Two of these variants are associated with an increase in risk for developing late-onset Alzheimer's disease (20,21). We also found that the activity of the proximal *APOE* promoter is upregulated by cAMP and retinoic acid, in astrocytic but not hepatic cells (22). The cAMP effect is mediated, in part, by interaction of factor AP-2α with two sites located in the *APOE* proximal region (22). The stimulatory effect of cAMP on *APOE* promoter in HepG2 cells involves AP-2α phosphorylation at Ser239 by protein kinase A (23). In this work, we investigate the existence of potential AP-2α-interacting factors which may modulate AP-2α activity. We have identified the oncoprotein DEK as an AP-2α-binding factor; DEK was found to enhance the effect of AP-2α on *APOE* promoter, probably enhancing the DNA binding of AP-2α.

MATERIALS AND METHODS

Recombinant protein expression

For the construction of the pcDNA3hisAP-2 vector, which expresses an N-terminal truncation of human AP-2α (deletion of the first 122 amino acids) with a poly-histidine extension at its N-terminal end (hisAP-2), a fragment was amplified by PCR using pTrcHisBAP-2 vector (23) as template, followed by cloning of the fragment into the pcDNA3 vector using the *Xho*I and *Hin*dIII cloning sites.

For purification of HisAP-2 from U-87 derived cell lines, identical amounts of nuclear protein from 25×10^6 cells (24) were diluted 50-fold with buffer D (0.2 M KCl, 20 mM Tris–HCl, pH 7.6, 10% glycerol, 2 mM $MgCl_2$ and protease inhibitors) and loaded onto a 200 µl Ni^{2+} column (Invitrogen Corporation) previously equilibrated in the same buffer. The column was washed with 20 vol of buffer D and eluted in 200 µl fractions with buffer D containing 500 mM imidazole.

Human recombinant AP-2α (AP-2r) was prepared as described previously (23). GST-DEK was an N-terminally truncated human DEK form containing 80 amino acids fused to GST kindly provided by Gerald Grosveld.

AP-2r affinity chromatography

Nuclear extracts from rat liver were prepared as described previously (25). The extracts were dialyzed against buffer B (20 mM Tris–HCl pH 7.6, 10% glycerol, 2 mM $MgCl_2$, 0.2 mM EDTA and 1 mM DTT) containing 0.2 M KCl and then passed through 30 ml of a heparin–Sepharose column

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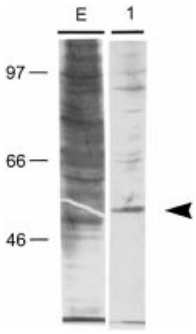

Figure 1. Purification of DEK by AP-2α affinity chromatography. A rat nuclear extract (E) was fractionated and enriched using an AP-2α affinity column (see Materials and Methods). The eluted fractions were pooled and subjected to SDS–PAGE, followed by silver staining (1). The band indicated by an arrow was identified as the rat homologue of human DEK by mass spectrometry.

(Amersham Pharmacia Biotech). The 0.5 M KCl buffer B eluted fractions were pooled, dialyzed against 0.1 M KCl buffer C (25 mM Tris–HCl pH 7.6, 12.5 mM $MgCl_2$, 20% glycerol, 1 mM DTT and 0.1% NP-40) and passed through an AP-2r–agarose column. This column was eluted with 1 M KCl buffer C. The AP-2r affinity column was constructed by coupling 250 mg of agarose (CNBr-activated Sepharose 4B) to 8 mg AP-2r (23) following the manufacturer's instructions (Amersham Pharmacia Biotech).

Cell lines, transfections, luciferase and β-galactosidase assays and electrophoretic mobility shift assays (EMSAs)

The cell lines U-87 MG (ATCC number: HTB-14) and HepG2 (ATCC number: HB-8065) were grown and transfected as described (23). For the selection of stable U-87 cell line expressing hisAP-2 protein, 10^6 cells were transfected with 20 µg of the pcDNA3hisAP2 vector and grown in selective medium (geneticin 0.4 mg/ml). After 2 days cells were plated in a limiting dilution in 24-well plates and AP-2α expression in single colonies was analysed by western blot using the anti-humanAP-2 antibody C-18 (Santa Cruz, CA). Luciferase and β-galactosidase activities were measured as described previously (23). EMSAs were carried out as reported (23). The CXX oligonucleotide sequence used in this work was the following: 5′-CTGTGCCTGGGGCAGGGGGAGAACA-3′.

Mass spectrometry

The identity of the purified band was determined following the same procedure described previously (23).

RESULTS

Identification of DEK as an AP-2α interacting factor using AP-2α-affinity chromatography

To identify potential AP-2α-interacting proteins, we carried out a two-step AP-2α-affinity chromatography purification using nuclear extracts from rat liver. A 50 kDa protein band was clearly enriched in the eluted fraction (Fig. 1). This band was excised from a silver stained SDS–polyacrylamide gel, subjected to in-gel tryptic cleavage and analysed by nanospray-ion trap MS/MS mass spectrometry. From the

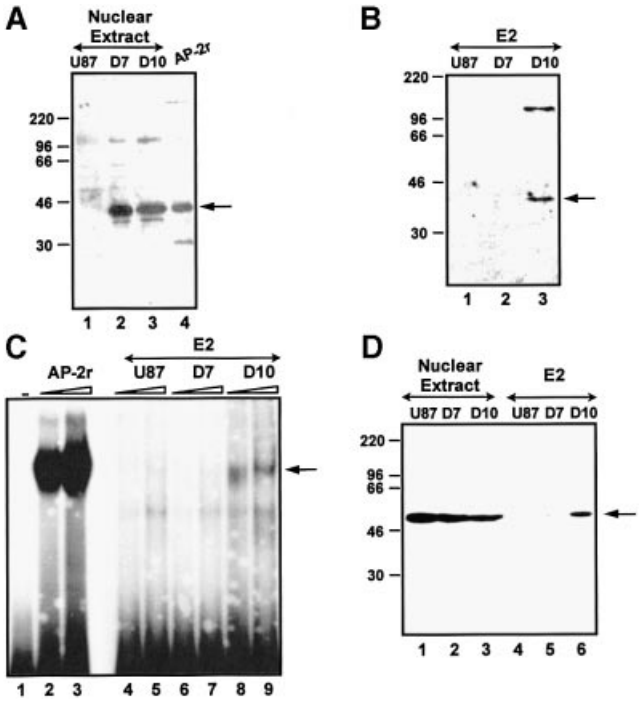

Figure 2. Interaction of hisAP-2 with human DEK in U-87, D7 and D10 astrocytoma cell lines. (**A**) Analysis of the expression of hisAP-2 in the stably transfected clones. Nuclear extracts (20 µg) from U-87, D7 and D10 were analysed by western blotting using C-18 antibody. As a positive control we used 1 µg of AP-2r. The arrow indicates the position of hisAP-2 band. (**B**) Purification of hisAP-2 from the stably transfected cell clones. Purified fractions (30 µl) from U-87, D7 and D10 were analysed by western blotting using the C-18 antibody. The arrow indicates position of hisAP-2. (**C**) DNA-binding activity of the purified fractions. EMSAs were carried out using 5 and 15 µl of the purified fractions from U-87, D7 and D10 and CXX probe. As a positive control 0.5 and 1 µg of AP-2r were used. The arrow indicates the position of the hisAP-2–DNA complex. (**D**) Co-purification of DEK with AP-2α. Ten micrograms of the original nuclear extracts and 40 µg of purified fractions from U-87, D7 and D10 were analysed by western blotting using a polyclonal antibody that recognizes the human DEK protein (33). The arrow indicates the position of the DEK band. In (A), (B) and (D), the molecular weight markers are indicated on the left.

total mixture of tryptic peptides, three of them (EPFTIAQGK, LLTNRPGTVSSLK and NVGQFSGFPFEK) were identified by a database search. All sequenced peptides showed a 100% identity to the human DEK protein, a 43 kDa protein initially discovered as a fusion partner of the CAN nucleoporin in a specific subtype of acute myeloid leukaemia (26). The identity between the sequenced peptides and the sequence of human DEK indicates that the purified protein is the DEK rat homologue, which has still not been cloned. We then investigated whether a physiologically relevant interaction between AP-2α and DEK occurs in human cells.

The oncoprotein DEK interacts with AP-2α in human cells

An N-terminal histidine-tagged AP-2α protein (hisAP-2) was stably expressed in the astrocytoma cell line U-87. Two highly expressing hisAP-2 U-87 clones, D7 and D10, were selected (Fig. 2A, lanes 2 and 3). From these two cell clones, we were only able to purify hisAP-2 from protein extracts from the D10 clone by using Ni^{2+} columns (Fig. 2B, lane 3), suggesting that the form of hisAP-2 expressed by D7 contained a

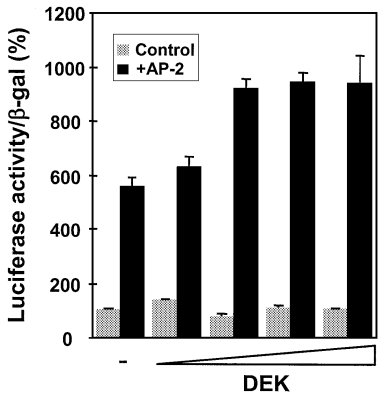

Figure 3. Effect of DEK on the transcriptional activity of AP-2α in HepG2 cells. 300 ng of the *APOE* promoter luciferase vector pXP2-227 were co-transfected with 300 ng of pcDNA3 (grey bars) or with an AP-2α expression vector (black bars) and increasing quantities of 0, 200, 400, 600 or 800 ng of the DEK expression vector combined with decreasing quantities of pcDNA3 of 800, 600, 400, 200 and 0 ng. Luciferase activity was corrected for the transfection efficiency taking into account the activity of 300 ng of β-galactosidase expression vector. Transfection assays were performed in triplicate and results are representative of at least three independent experiments; data are expressed as mean ± SEM.

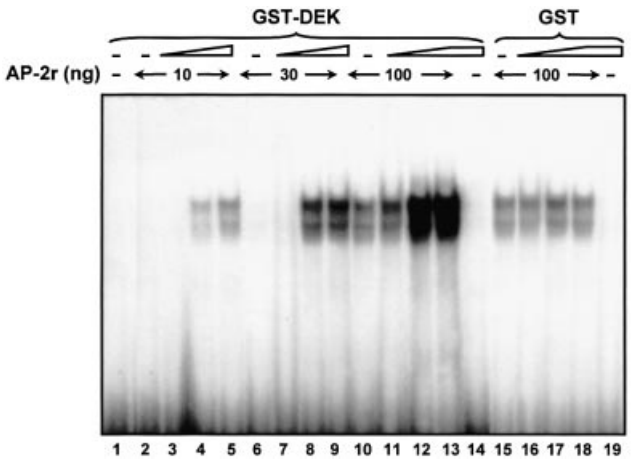

Figure 4. Effect of DEK on binding of AP-2r to DNA *in vitro*. CXX probe was incubated with 10 (lanes 2–5), 30 (lanes 6–9) or 100 ng (lanes 10–13) of AP-2r in the presence of either 20 (lanes 3, 7 and 11), 100 (lanes 4, 8 and 12) or 500 ng (lanes 5, 9 and 13) of GST-DEK. As negative controls, CXX probe was incubated with 100 ng of AP-2r in the presence of either 20 (lane 16), 100 (lane 17) or 500 ng (lane 18) of GST, and 500 ng of GST-DEK (lane 14) and GST (lane 19) were incubated with the probe in the absence of AP-2r.

non-functional protein due to some mutation or deletion in the Ni^{2+}-binding domain. Consistently, the fractions purified from protein extracts from D7 did not present any noticeable DNA binding activity, whereas this activity was detected in the fractions eluted from Ni^{2+} columns loaded with clone D10 extracts (Fig. 2C, compare lanes 6 and 7 with 8 and 9). We therefore used the D7 clone as an internal control in subsequent experiments. An unidentified 110 kDa band co-purified with AP-2α and was recognised by the anti-AP-2 antibody (Fig. 2B, lane 3). A band of similar molecular weight has been described by other authors using anti-AP-2α antibodies (27,28).

We next investigated whether DEK co-purified with hisAP-2. The nuclear expression levels of DEK in the two clones, D7 and D10, were similar to those of the original U-87 cell line (Fig. 2D, lanes 1–3). DEK was clearly detected in the AP-2α-enriched fractions when nuclear extracts from clone D10 where subjected to Ni^{2+}-affinity chromatography (Fig. 2D, lane 6). In clear contrast, DEK was absent in the eluates from either U-87 or clone D7 (Fig. 2D, lanes 4 and 5). These data strongly suggest that DEK interacts with AP-2α in cell cultures.

DEK increases transcriptional activity of AP-2α in HepG2 cells

To investigate the physiological relevance of the DEK-AP-2α interaction, we tested whether DEK could modulate AP-2α transcriptional activity. We co-transfected increasing amounts of a DEK expression vector with a constant amount of an AP-2α expression vector and an *APOE* promoter-luciferase reporter vector [from –227 to +1 *APOE* promoter sequence (22)] in the hepatoma cell line HepG2. According to previously reported results (22,23,29), AP-2α transactivated *APOE* promoter in these cells (Fig. 3). The addition of increasing amounts of DEK enhanced AP-2α activity in a dose-dependent manner (Fig. 3, black bars). AP-2α was directly involved in this effect, since overexpression of DEK alone did not have any effect on *APOE* promoter activity (Fig. 3, grey bars). Taken together, these results suggested that DEK plays a role as co-activator of the transcriptional activity of AP-2α.

DEK enhances binding of AP-2α to DNA

We then examined whether a recombinant preparation of GST-DEK affected the formation of AP-2α–DNA complexes by EMSAs. As shown in Figure 4, while *in vitro* DNA binding activity of small amounts of AP-2r (30 ng) was undetectable, formation of AP-2r–DNA complexes could be observed in the presence of GST-DEK. This effect of GST-DEK appeared to be specific since GST did not enhance the DNA-binding activity of AP-2α (Fig. 4). The stimulatory effect of GST-DEK was dose dependent (Fig. 4), and we were unable to observe the formation of DNA complexes with GST-DEK alone (Fig. 4). In addition, the presence of GST-DEK did not produce a significant increase in the mobility of the AP-2α–DNA complex. In our assay conditions we have been unable to detect a supershift band with an anti-humanDEK antibody (30) (data not shown) suggesting that DEK is not present in the complex; it is also possible that DEK is not recognised by the antibody in the presence of bound AP-2α due to some kind of steric hindrance. Taken together, our results suggest the existence of a physiologically relevant interaction between the oncoprotein DEK and the transcription factor AP-2α; DEK enhances AP-2α transcriptional activity by a mechanism that appears to involve an enhancement in AP-2α–DNA binding.

DISCUSSION

To the best of our knowledge, this is the first report that suggests a role for the oncogene DEK as co-activator of a transcription factor. DEK contains a SAP (after SAF-A/B, Acinus and PIAS) DNA-binding motif which is involved in chromatin remodelling and transcription (31). Coincidentally,

several SAP family members, such as Miz1 (32), alter the DNA binding activity of transcription factors, modifying their transcriptional activity. Since DEK is known to bind condensed chromosomes (33) and chromatin (30,34), altering the nucleosomal DNA topology and reducing the chromatin replication efficiency (35), it is possible that DEK produces a change in chromatin conformation that increases the DNA binding of AP-2α. The AP-2α binding site on *APOE* promoter (TGGGGCAGGG) is very similar to the known DEK binding site in the HIV-2 promoter, which is a pets sequence (TTGGTCAGGG) (30). Although DEK does not appear to bind to the CXX probe *in vitro*, it is possible that, upon a possible *in vivo* binding to DNA, DEK may act as a recruitment factor for AP-2α. In *Saccharomyces cerevisiae*, the expression of Pho5 is controlled by a similar 'cooperativity' mechanism. In this case, the Pho4 transcriptional activator is cooperatively recruited to the active sites by the homeoprotein Pho2, a protein that binds very weakly to DNA in the absence of Pho4 (36).

However, DEK appears to enhance the interaction of AP-2α with DNA without affecting the rate of migration of this complex, and no evidence was found suggesting a direct interaction of DEK with DNA. Thus, DEK behaves similarly to Miz1, another SAP protein, which enhances DNA binding of the transcription factor Msx2 without forming ternary complexes (32). Our results suggest the existence of a transient interaction in which DEK affects the conformation of AP-2α, enhancing its DNA binding. A similar 'hit and run' mechanism has been demonstrated for the effect of Phox 1 over DNA binding of the serum response factor (SRF); Phox 1 was proposed to lower an activation energy barrier, enhancing the rate of stable SRF–DNA complex formation (37).

The interaction of AP-2α with DEK was demonstrated using an N-terminally truncated AP-2α fragment. This suggests that the interaction domain of AP-2α is located in the C-terminal moiety, which includes the DNA binding and dimerization domains. Both domains are highly conserved between all members of the AP-2 family (5,38) suggesting that DEK may be interacting with other AP-2 family members.

It has recently been suggested that cardiac, neural crest and neural tube defects in mice lacking Cited2 are produced because this protein plays a role as AP-2α co-activator (39). Also, over-expression of some co-activators, such as PC4 and PARP, partially rescue the malignant phenotype of PA-1 cells transformed by AP-2α via a mechanism that involve an effect of these co-activators over other transcription factors (27,40). Since DEK can partially revert the transformation-prone phenotype of cells from patients with ataxia-telangiectasia (41), it is reasonable to think that DEK may rescue this phenotype due to the action of DEK-dependent transcription factors, such as AP-2α. In this sense, we can speculate that the oncogenic activity of the chimera DEK-CAN (26) may be due to the inhibition of the interaction with AP-2α and other transcription factors, provoking, as a result, cell transformation.

It has been demonstrated that DEK is an oncogene associated with human hepatocellular carcinogenesis (42). The DEK oncogenic properties and the co-expression of DEK and AP-2α in several tissues such as spleen, thymus, kidney and brain (6,26) suggest that DEK could be acting in a coordinate way with AP-2α in the cell cycle regulation.

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